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Kinetics of measles antibody by hemagglutination inhibition assay in children in south-west and north-central Nigeria

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SUMMARY

Objectives: We investigated the antibody level of children against wild measles virus in view of recurrent measles epidemics, in order to provide information on immunization status for health policies and for the global measles mortality reduction initiative.
Methods: Two hundred and seventy-three children between the ages of 10 months and 13 years were recruited for this study from three hospital facilities in south-west and north-central Nigeria. Serum samples were collected from February to July 2009, and laboratory examination commenced in August of the same year. Measles hemagglutinin (HA) antigen was prepared by culturing the measles vaccine virus strain (Edmonston-Zagreb) in a vero/hSLAM cell line. Serum samples were treated to get rid of potentiating factors, non-specific inhibitors, and agglutinins before the HA/hemagglutination inhibition (HI) procedure.
Results: Out of the 175 children vaccinated in Ibadan, 60 (34.3%) had an antibody level not sufficient to protect against measles infection. Likewise, 12 (25.0%) vaccinated children from Ilorin had an antibody level not sufficient to protect against measles infection. There was no significant difference in the level of protection between the children in Ibadan and Ilorin ($p > 0.05$). The geometric mean titer (GMT) was 53.83 for males and 48.64 for females. There was no significant difference between the GMTs of females and males in both locations ($p > 0.05$). Also, no significant difference was observed in the GMTs of children in both locations ($p > 0.05$).
Conclusions: Of the vaccinated children, 157 (57.5%) developed protective measles virus HI antibody, which is not enough to maintain protective herd immunity. Hence there is a need for catch-up and follow-up vaccination programs, especially in rural areas and places with difficult terrains, in order to reduce measles mortality.

1. Introduction

Measles is a major childhood problem and of serious concern in Africa, Latin America, Europe, Southeast Asia, and the Eastern Mediterranean.[1] The Health Protection Agency (HPA) in the UK revealed that 496 laboratory confirmed cases were reported in England and Wales up to the end of May 2011, surpassing the annual 2010 total of 374 cases.[2] In France, more than 7500 cases were reported from January through March 2011. Cases have been reported from 38 countries across the region, including outbreaks in Spain, Serbia, Macedonia, and Turkey, among others. More than 10 000 cases were reported from countries in the European Economic region in the first 4 months of 2011.[3]

In Africa, about 13 million cases and 650 000 deaths are estimated to occur annually, and Sub-Saharan Africa to which Nigeria belongs is one of the regions of the globe with the highest measles morbidity and mortality.[4] Despite the comprehensive measles reduction strategy and partnership with the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), and other international organizations in Africa, certain high burden countries continue to face recurrent epidemics.[5] Nigeria has a high childhood mortality rate, with measles being one of the leading causes.[6] Measles routine immunization has been low in Nigeria and this has been responsible for the frequent measles outbreaks observed in different parts of Nigeria. However, between 2005 and 2008, there was a general awakening on measles vaccination, which culminated in an aggressive routine measles immunization program with catch-up and follow-up vaccinations.

The objective of this study was to ascertain the level of protection in children following the catch-up measles vaccination

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campaign that was embarked upon in Nigeria in 2005 and 2006 (in the southern and northern states, respectively) and the nationwide follow-up campaign in 2008.

2. Materials and methods

2.1. Study design

Three hospitals were chosen for this study: Adeoyo Maternity Teaching Hospital and Oni Memorial Children's Hospital, both in Ibadan, Oyo State (south-west), and Abanise-lolu Children's Hospital in Adeta–Ilorin, Kwara State (north-central). This study was conducted in two states of the federation between February and July 2009. This was done to compare the immune status of children in parts of south-west and north-central Nigeria.

2.2. Study population

A total of 273 children between the ages of 10 months and 13 years, from different socio-economic backgrounds, were recruited into the study. Of the 205 (75.1%) children from Ibadan, 99 (36.3%) were from Adeoyo Maternity Teaching Hospital and 106 (38.8%) from Oni Memorial Children's Hospital. Sixty-eight (24.9%) children were from Abanise-lolu Children's Hospital. Informed parental consent was sought before a blood sample was collected. Details of the vaccination history were obtained through questionnaire and/or from parental recall. The first dose of measles vaccine is given at 9 months, with a booster dose expected at 12–15 months of age, but this is usually ignored. The actual age at which individual children were subsequently vaccinated was not recalled by their parents.

2.3. Sample collection and handling

Between 3 and 5 ml of blood were collected in plain tubes through venipuncture. The blood was allowed to clot and was centrifuged at 3000 rpm for 10 min. Serum was carefully removed with a Pasteur pipette and transferred to sterile labeled cryovials. These were labeled with a sample identification number, date of collection, and specimen type and were then stored at −20 °C until the representative sample size was obtained.

2.4. Virus culture

The measles virus hemagglutinin antigen (MV HA Ag) was prepared from the Edmonston-Zagreb vaccine strain obtained from the Public Health Centralized Immunization Clinic of the University College Hospital, Ibadan. The vaccine was manufactured by the Serum Institute of India Ltd (Pune, India). Growth and maintenance media were made from Eagle's minimum essential medium (Sigma-Aldrich, USA) supplemented with 10% and 2% fetal calf serum, respectively. A vero/hSLAM cell line was grown in two T75 flasks and inoculated with 0.4 ml of reconstituted measles vaccine virus. These are vero cells that have been transfected with a plasmid encoding the gene for the human signaling lymphocyte activation marker (hSLAM).[7] On the fifth day post-inoculation, the cells were harvested after the cytopathic effects (CPE) of stellate bodies and syncytia were observed. The polyethylene glycol (PEG) method of virus purification was carried out overnight at +4 °C and then 5 μl of tween80 detergent was added on ice and vigorously shaken. Two milliliters of ether were added and spun for 20 min at 3000 rpm in a cold centrifuge. Purified MV HA Ag was collected from the interphase and tested for hemagglutination activity.[8–11]

Table 1
Distribution of the immune status of children in the study population in selected hospitals of Ibadan and Ilorin

Immune status	Ibadan	Ilorin	Total
Protected	121 (59.0%)	36 (52.9%)	157 (57.5%)
Unprotected	84 (41.0%)	32 (47.1%)	116 (42.5%)
Total	205 (100.0%)	68 (100.0%)	273 (100.0%)

2.5. Hemagglutination inhibition assay (HIA)

The hemagglutination test was first carried out using the MV HA Ag to obtain 1 HA unit, from which the 4 HA units (standard working concentration) was obtained. The 1 HA titer was 320. The HIA was then performed by a modification of the method used by Gershon and Krugman.[12] Serum was heat-inactivated at 56 °C for 30 min to get rid of complements and potentiating factors. Non-specific inhibitors of hemagglutination and non-specific agglutinins were removed from test sera by the addition of 0.1 ml of each serum to 0.4 ml borate saline and 0.5 ml of a 25% suspension of acid washed kaolin. The mixture was spun at 1200 rpm for 10 min, and 25 μl of packed patas monkey (*Erythrocebus patas*) erythrocytes was added to remove non-specific agglutinins. Two serial dilutions of the treated sera were done starting from 1:10 through 1:320. Then 0.025 ml of 4 HA units of antigen was added to each well of the serially diluted sera in U-bottom 96-well microtiter plates. Each well received 0.025 ml of a 0.5% suspension of monkey erythrocytes. Plates were shaken and incubated for 1 h at 36 °C. Complete inhibition of agglutination at 1:40 dilution of serum was considered as the protection level.[8,13,14] Serum from a previously vaccinated individual was used as control.

2.6. Statistical analysis

Statistical analysis was carried out with SPSS version 20.0 to test for equality of the means using the independent *t*-test.

3. Results

In this study, 157 (57.5%) children were found to be protected and 116 (42.5%) were found to be unprotected, as shown in Table 1.

Two hundred and twenty-three (81.7%) children were vaccinated and 50 (18.3%) were not. Out of the 205 children from Ibadan, 175 (85.4%) were confirmed to be vaccinated, while 48 (70.6%) were vaccinated in Ilorin (Table 2). Out of the 175 children vaccinated in Ibadan, 60 (34.3%) had an antibody level not sufficient to protect against measles infection. Likewise, 12 (25.0%) children vaccinated in Ilorin had an antibody level not sufficient to protect against measles infection (Table 2). There was no significant difference in the level of protection between children in Ibadan and Ilorin ($p > 0.05$).

The mean age of children was 4.2 ± 2.7 years for females and 4.7 ± 2.5 years for males. The mean age of male and female children was not significantly different in both locations ($p > 0.05$). One

Table 2
Distribution of the vaccination status of children with respect to immune status

Vaccination status/immune status	Ibadan	Ilorin
Vaccinated		
Protected	115 (65.7%)	36 (75.0%)
Unprotected	60 (34.3%)	12 (25.0%)
Total	175 (100.0%)	48 (100.0%)
Nonvaccinated		
Protected	6 (20.0%)	0 (0.0%)
Unprotected	24 (80.0%)	20 (100.0%)
Total	30 (100.0%)	20 (100.0%)

Table 3
Distribution of measles virus hemagglutination inhibition titers according to the gender of the children at selected hospitals in Ibadan and Ilorin, Nigeria

Variables	Ibadan	Ilorin	Combined
GMT			
Males	51.44	60.54	53.83
Females	49.70	45.16	48.64
MV HI titer			
10	43 (20.9%)	23 (33.8%)	66 (24.2%)
20	41 (20.0%)	9 (13.2%)	50 (18.3%)
40	58 (28.3%)	18 (26.5%)	76 (27.8%)
80	47 (22.9%)	8 (11.8%)	55 (20.1%)
160	13 (6.3%)	7 (10.3%)	20 (7.3%)
320	3 (1.5%)	3 (4.4%)	6 (2.2%)
Total	205 (100%)	68 (100%)	273 (100%)
Gender			
Males	104 (50.7%)	37 (54.4%)	141 (51.6%)
Females	101 (49.3%)	31 (45.6%)	132 (48.4%)
Total	205 (100.0%)	68 (100.0%)	273 (100.0%)

GMT, geometric mean titer; MV, measles virus; HI, hemagglutination inhibition.

hundred and four (50.7%) children in Ibadan were males and 101 (49.3%) were females, while 37 (54.4%) of the children from Ilorin were males and 31 (45.6%) were females, as shown in Table 3.

There was no significant difference in the geometric mean titer (GMT) of male and female children in each location and both locations combined ($p > 0.05$). One hundred and seventy-four (63.7%) children in the study were between 1 and 5 years of age, 13 (4.8%) were aged less than 1 year, and 86 (31.5%) were older than 5 years, with the oldest being 13 years of age, as shown in Table 4.

4. Discussion

This study examined the level of measles hemagglutinating antibody in children in three selected hospitals in the south-west and north-central geopolitical regions of Nigeria. The HIA is a classical technique that has been employed in prevalence studies and has good correlation with ELISA and neutralization techniques. It reflects the real level of the population's antibodies.[15]

Results obtained in this study show that a large percentage of children aged under 5 years in the two states studied were not protected from measles infection. This is a matter of concern, as this age group is an indication of the vulnerability of children to measles infection. Measles is the fifth major cause of childhood mortality in Nigeria.[6] Of particular interest in this study is the relatively large number of vaccinated children in both states and locations who were found not to be protected. While this could be as a result of secondary vaccine failure, it could also be due to unsubstantiated wrong information from parents and guardians about the true vaccination status of their children. There was no significant difference in the level of protection between the children in Ibadan and Ilorin ($p > 0.05$). We also observed in this study that the antibody level rose according to the age of the children. This is in agreement with the study of Sakata and Sugiura[16] who showed that the level of antibody directed against the hemagglutination protein was 1:32 for the first weeks of infection, which increased to 4:32 over several years (dilution factor 8 as against our dilution factor 10). There was no significant difference in the GMTs of males and females in the two locations. Our observations in this study showed that children with a previous infection from a past epidemic developed a high antibody profile, with titers as high as 1:160. Hamid and colleagues observed that the number of protected-unprotected children with a high antibody level was higher in those parts of Nigeria where there had been measles epidemics.[17] Measles has been known to confer life-long immunity post infection.[18]

Table 4
Frequency distribution of the age of children at selected hospitals in Ibadan and Ilorin, Nigeria

Age group	Ibadan	Ilorin	Combined
<1 year	9	4	13
1–5 years	137	37	174
>5 years	59	27	86

Table 2 shows that of the 273 children studied, 175 (85.4%) and 48 (70.6%) were vaccinated in the selected hospitals in Ibadan (south-west) and Ilorin (north-central) geopolitical regions. This is in agreement with vaccination coverage of 71–82% also reported nationally.[19]

This was a retrospective study. The immune response in this study may be due to routine vaccination, or the catch-up vaccination of 2005 and 2006, as well as follow-up of 2008. It may therefore be inferred that the remarkable level of protection observed in this study may be the after-effect of the campaign. As shown in Table 2, only six (12.0%) of the unvaccinated children were protected as a result of recovery from natural infection. Further studies are needed to confirm this. In this study, we observed that 50 (18.3%) and 66 (24.2%) children had unprotective titers of 20 and 10, respectively (Table 3). This could be due to vaccine failure due to a break in the cold chain, or malnutrition. Past studies have shown that a break in the vaccine cold chain results in the inability to develop measles protective antibodies.[20]

In conclusion, measles immunization can serve as a proxy indicator for access to basic health services for children aged <5 years.[21] We recommend that routine measles vaccination be improved in Nigeria in order to reduce the incidence of measles outbreaks.

4.1. Challenges

This study was carried out after the National Programme on Immunization (NPI) vaccination campaign, so we could not conduct a pre-NPI sero-survey. We had a small number of serum samples from Ilorin, Kwara State because we could not collect them ourselves due to distance and logistics problems. We lacked materials to embark on a more sophisticated and modern laboratory technique (plaque reduction virus neutralization) so we relied on the HA/HI technique having expended a lot of resources in a resource-limited country. We received no grant or financial assistance whatsoever, but used personal funds.

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